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Analysis of fingerprint samples, testing various conditions, for forensic DNA identification

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ABSTRACT

Fingerprints can be of tremendous value for forensic biology, since they can be collected from a wide variety of evident types, such as handles of weapons, tools collected in criminal cases, and objects with no apparent staining. DNA obtained from fingerprints varies greatly in quality and quantity, which ultimately affects the quality of the resulting STR profiles. Additional difficulties can arise when fingerprint samples show mixed STR profiles due to the handling of multiple persons. After applying a tested protocol for sample collection (swabbing with 5% Triton X-100), DNA extraction (using an enzyme that works at elevated temperatures), and PCR amplification (AmpFlSTR® Identifiler® using 31 cycles) extensive analysis was performed to better understand the challenges inherent to fingerprint samples, with the ultimate goal of developing valuable profiles (≥50% complete). The impact of time on deposited fingerprints was investigated, revealing that while the quality of profiles deteriorated, full STR profiles could still be obtained from samples after 40 days of storage at room temperature. By comparing the STR profiles from fingerprints of the dominant versus the non-dominant hand, we found a slightly better quality from the non-dominant hand, which was not always significant. Substrates seem to have greater effects on fingerprints. Tests on glass, plastic, paper and metal (US Quarter dollar, made of Cu and Ni), common substrates in offices and homes, showed best results for glass, followed by plastic and paper, while almost no profiles were obtained from a Quarter dollar. Important for forensic casework, we also assessed three-person mixtures of touched fingerprint samples. Unlike routinely used approaches for sampling evidence, the surface of an object (bottle) was sectioned into six equal parts and separate samples were taken from each section. The samples were processed separately for DNA extraction and STR amplification. The results included a few single source profiles and distinguishable two person mixtures. On average, this approach led to two profiles ≥50% complete per touched object. Some STR profiles were obtained more than once thereby increasing the confidence.

1. Introduction

Numerous items recovered from crime scenes that are routinely examined by forensic biology laboratories are most commonly associated with homicides, felony assaults, sexual assaults, robberies, and burglaries. These items may include handled objects, such as weapons or tools that were touched by one or multiple persons. DNA recovered from these items most likely originated from skin cells. Skin is remarkably dynamic; cells move from the basal to the upper epidermal layer of the skin as they mature. During this process their cytoplasm condenses and becomes highly keratinized and their nuclear DNA becomes fragmented through apoptosis [1]. Daily, thousands of skin cells are shed and transferred onto items the skin comes in contact with [2–5]. A large portion of these skin cells/flakes do not contain nuclei. However, forensically informative STR profiles can be obtained from touched samples [6,7]. Many possible sources of DNA may contribute to these samples. In part, some of the condensed cells still have nuclear DNA [6]. Skin flakes may serve as vectors for extracellular DNA transferred from other body surfaces (eye, nose, and mouth) as well as from sebaceous and sweat glands [4,8–11]. Therefore, cells from diverse areas may be present in so-called 'touch' samples (reviewed in [10]). Consequently, it is likely to expect great variation in quality and quantity of DNA from such evidence [7,12,13]. In addition, external conditions may affect the DNA recovery from touched evidence, such as (i) a time lag between deposition and evidence collection [14], (ii) surface of substrate, and (iii) the length of time of the contact to transfer cellular material (reviewed in [10]). It was shown, despite poor DNA quantities, a routine contact for as little as 10 s can lead to 3 ng of DNA [15,16]. Understanding the challenges inherent to touched DNA samples, and designing a work flow to address them, will improve the quality of the STR profiles obtained from such samples. This may be of crucial importance to some cases, where touched evidence may be the only available source of information.

In casework, touched DNA samples are often difficult to interpret because of low DNA amounts, DNA degradation and/or the contribution of DNA from multiple individuals. DNA mixtures are likely because

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multiple individuals could have handled items found at crime scenes prior to their collection as an item of evidence. Routinely used methods of sample collection [16,17] tend to generate admixed DNA samples, which only can be used for comparisons and therefore do not provide as much investigative information as single source or deconvoluted mixture DNA profiles.

In this study, we used a tested protocol [12] for sample collection (swabbing using 5% Triton X-100), DNA extraction (using an enzyme that works at elevated temperatures), and PCR amplification (AmpF*l*STR® Identifiler® using an increased cycle number: 31) in an attempt to overcome some of the inherent challenges in developing STR profiles from touched items. We tested fingerprints stored for different time periods and on commonly touched substrates. In addition, we assessed mixtures by sampling multiple sections of an item, generating several samples that are processed separately [18]. To our knowledge, this is the first time that this approach was tested on touched three person mixtures.

2. Material and methods

2.1. Sample collection

This study was approved by the New York City Department of Health and Mental Hygiene Institutional Review Board that oversees research involving human subjects for the Office of Chief Medical Examiner (OCME) (IRB# 12-058). Volunteers who participated in this study read and signed the consent form before donating fingerprints. Each volunteer was assigned with a code to anonymize the sample. Six volunteers contributed to this study.

Volunteers were asked to refrain from washing their hands for at least 2 h prior to sampling for all experiments. Fingerprint samples were taken by pressing right and left thumbs for a few seconds (~3 s) on various substrates that are commonly used [glass (microscope slide, 25 mm × 75 mm, of which 19 mm were frosted for labeling), plastic (sheet protector made of polypropylene, Avery®), paper (office paper), and metal (a US Quarter dollar, 24.26 mm in diameter, made of 91.67% Cu and 8.33% Ni)]. Prior to collecting fingerprints, the substrates were decontaminated by immersing the entire object in 10% bleach, followed by water, and 70% ethanol. The paper was UV irradiated for 30 min in the NuAire biosafety cabinet (NuAire, Plymouth, MN). Fingerprints on plastic, paper, and metal were stored in closed, decontaminated boxes for 3 days, to allow for an effect on the deposited cellular material and to mimic casework samples. Fingerprints on microscope slides were stored at room temperature in closed and open decontaminated boxes in a laboratory, for the following time periods: 1, 3, 10, 20, and 40 days. For the mixture study, the body of an empty beer bottle (amber glass: height of bottle: 23 cm, height of trunk: 12 cm, diameter of trunk: 6 cm) was held in the hand of each volunteer for 60 s, allowing for the possibility of both the palm and fingers to touch the bottle. No further guidance was given. The trunk of the beer bottle, from which the label was removed, was initially etched into six equivalent sections; each section measured 6 cm × 6.3 cm. Three people held the bottle consecutively with their dominant hand. The order of people touching the bottle was alternated. The beer bottle was kept unpackaged, on a laboratory bench at room temperature and was swabbed one day following touching.

Fingerprints were swabbed under the Olympus SZX-16® stereomicroscope (Olympus of the Americas, Central Valley, PA, USA) using a small portion of a sterile, UV irradiated cotton swab (Dynarex, Orangeburg, NY), held with reverse clamp tweezers (Dumont N5 dissecting tweezers, Ted Pella, Stockholm, Sweden), and moistened with 4 μl of 5% Triton X-100 (Sigma-Aldrich, USA) [19]. A clean substrate was swabbed alongside each batch of samples as negative control. If this substrate control tested positive for DNA the samples of this batch would be discarded.

2.2. DNA extraction and quantification

DNA was isolated from swabbed samples using prepGEM® Tissue extraction kit (Zygem, Corporation Ltd., New Zealand) following the manufacturer's instructions. Briefly, swabs were incubated in 20 μl of prepGEM® tissue extraction mixture for 15 min at 75 °C, followed by 5 min at 95 °C using a GenAmp 9700 thermal cycler (Life Technologies, Applied Biosystems, Foster City, CA) [12]. The extracted DNA was quantified using *Alu*-based real-time PCR for human DNA adapted from Nicklas and Buel [20]. Two microliter of extracted DNA was used as template in a 25 μl reaction using SYBR-Green (Life Technologies Molecular Probes, Grand Island, NY) and Ampli-*Taq* Gold (Life Technologies) on a Rotorgene™ Q (Qiagen, Valencia, CA). Samples were quantified for the time course study as indicated and for the three-person mixtures. For the comparison of left versus right fingerprints all samples were used regardless if quantified or not, since right and left hand were always treated equally. The samples were not quantified for the tests of various substrates.

2.3. Amplification of STR loci

All samples were amplified using AmpF*l*STR® Identifiler® PCR Amplification Kit for 31 cycles (Life Technologies Applied Biosystems, Foster City, CA), following the low-template DNA testing protocol validated at the OCME [16]. The DNA of one sample was extracted in 20 μl. If the sample was quantified (2 μl), 5 μl were used as template for the STR amplification, which was performed in triplicate (3 x 5 μl = 15 μl). If the sample was directly used for STR amplification, 6 μl were used as template (3 × 6 μl = 18 μl). Besides the DNA template, each STR amplification contained 2.5 μl Primer Mix, 5 μl Reaction Mix, and 0.5 μl Ampli*Taq* Gold DNA Polymerase (5 U/μl). A negative control was used for each STR amplification batch, and if positive, the entire batch was disregarded.

2.4. Capillary electrophoresis and analysis

Amplified PCR products were separated on a 3130*xl* Genetic Analyzer (Life Technologies Applied Biosystems, Foster City, CA) at 3 kV for 20 s. Samples with overblown signals were reinjected at 1 kV for 22 s, and samples with low signals were reinjected at 6 kV for 30 s. Data analysis was performed using GeneMapper v. 4.0 software (Life Technologies Applied Biosystems). The peak amplitude threshold in GeneMapper was set to 75 RFU detection. Peak ratio cut off value for tetra-nucleotide markers was set to 0.1.

The STR amplification was carried out in triplicates. Alleles that were present in at least two of three amplifications were considered part of the consensus profile. Only these alleles were assigned to the DNA profile of the sample [16]. Using in-house developed interpretation guidelines for single source and mixed low template DNA samples, the donor's DNA profile was assigned [16]. For single-source samples, heterozygous alleles were determined based on the two tallest peaks observed in at least two of three amplifications, whereby the smaller peak must be ≥50% of the larger peak. Homozygous alleles must appear in all three amplifications, in order to be assigned to a profile. Any additional peaks, repeating or not, must be <30% of the major peak in order to call the locus a true homozygote [16]. For mixed samples, only clear major components were used. Heterozygous alleles were assigned when they appeared in all three amplifications and showed a peak balance greater than or equal to 50% in two out of the three replicates. Homozygous alleles must also appear in all three amplifications and must be clearly the major peak, while the minor peaks were <30% [16].

Identifiler® kits amplify 15 autosomal loci plus Amelogenin, for a maximum of 30 autosomal alleles per donor. New York and the U.S. require a minimum of six core loci for upload to SDIS (State DNA Index System) and ten to NDIS (National DNA Index System). This study used percentages in order to compare the outcomes. The profile